

Amendments to the Specification:

Please replace the last filed Sequence Listing with the paper and computer-readable forms of the Sequence Listing submitted concurrently herewith.

On page 1, please insert the following section immediately after the title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the U.S. National Stage of PCT/EP2004/0013780, filed December 3, 2004, and claims the benefit under 35 U.S.C. § 120 of PCT/EP03/13656, filed December 3, 2003 and designating the U.S., which is hereby incorporated herein in its entirety by reference.

Please replace the paragraph which begins as the last paragraph on page 32 and continues on page 33 with the following paragraph:

Many examples of MTSs, natural and synthetic, are known in the art. An MTS may be a simple amino acid repeat, for example a cationic peptide containing eleven arginines (SEQ ID NO:16) RRRRRRRRRR (Matsushita *et al.*, 2001, *J. Neurosci.*, 21, 6000-6007). Another cationic MTS is a 27 amino acid long transportan (SEQ ID NO:17) (GWTLNSAGYL LGKINLKALA ALAKKIL) (Pooga *et al.*, 1998, *FASEB J.*, 12, 67-77). It is very likely that such peptides, for their penetration of the cell, exploit the asymmetry of the cellular plasma membrane where the lipid monolayer facing the cytoplasm contains anionic phospholipids (Buckland & Wilton, 2000, *Biochim. Biophys. Acta/Mol. Cell. Biol. Of Lipids*, 1483, 199-216). Many proteins contain subunits that enable their active translocation across the plasma membrane into cells. Examples of such subunits are the basic domain of HIV-1 Tat<sub>49-57</sub> (SEQ ID NO:18) (RKKRRQRRR) (Wender *et al.*, 2000, *Proc. Natl. Acad. Sci. USA*, 97, 13003-13008), Antennapedia<sub>43-58</sub> (SEQ ID NO:19) (RQIKIWFQNR RMKWKK) (Derossi *et al.*, 1994, *J. Biol. Chem.*, 269, 10444-10450), the Kaposi Fibroblast Growth Factor MTS (SEQ ID NO:20)

(AAVALLPAVL LALLAP) (Lin *et al.*, 1995, *J. Biol. Chem.*, 270, 14255-14258); the VP22 MTS (Bennet, Dulby & Guy, 2002, *Nat. Biotechnol.*, 20, 20; Lai *et al.*, 2000, *Proc. Natl. Acad. Sci. USA*, 97, 11297-302); homeodomains from the *Drosophila melanogaster* Fushi-tarazu and Engrailed proteins (Han *et al.*, 2000, *Mol Cells* 10, 728-732). It was shown that all these positively charged MTSs are able to achieve cell entry by themselves and as fusions with other proteins like GFP (Zhao *et al.*, 2001, *J. Immunol. Methods*, 254, 137-145; Han *et al.*, 2000, *Mol Cells*, 10, 728-732), Cre recombinase (Peitz *et al.*, 2002, *Proc. Natl. Acad. Sci. USA*, 4489-4494) in an energy-independent manner. However, the fusion is not necessarily required for protein transport into the cell. A 21-residue peptide carrier Pep-1 was designed (SEQ ID NO:21) (KETWWETWWTEWSQPKKKRKV) which is able to form complexes by means of non-covalent hydrophobic interactions with different types of proteins, like GFP, b-Gal, or full-length specific antibodies. These complexes are able to efficiently penetrate cell membranes (Morris *et al.*, 2001, *Nature Biotechnol.*, 19, 1173-1176). The list of MTSs can be continued and, in general, any synthetic or naturally occurring arginine-rich peptide can provide the signal protein of the invention with the ability of entering plant cells (Futaki *et al.*, 2001, *J. Biol. Chem.*, 276, 5836-5840).

Please replace the first paragraph on page 36 with the following paragraph:

The *lacI* coding sequence was PCR-amplified from E. coli strain XL1-Blue with primers olac1 (SEQ ID NO:1) (5'-gaccatggaaccagtaacgttatacatg-3') and olac2 (SEQ ID NO:2) (5'-cactg cagtcactgccccgcttccag-3'), adding an NcoI and a PstI restriction site to the ends. The coding sequence was fused to the plasmid *rrn16* promoter by insertion into the corresponding restriction sites of vector pKCZ (Zou *et al.*, 2003), replacing the *aadA* coding sequence, resulting in plasmid pICF9851. A modified version of the *rrn16* promoter containing a lac operator site between the -10 and -35 boxes was made by inverse PCR with primers olac3 (SEQ ID NO:3) (5'-acgattgtgagcggataacaatatattctgtggagcgaac-3') and olac4 (SEQ ID NO:4) (5'-caatcccacgagcct cttatc-3') from plasmid pICF7341 which contains the cloned promoter sequence amplified by PCR from tobacco DNA. The modified promoter was excised from the resulting plasmid with Sall and BamHI. A further fragment consisting of the smGFP coding sequence from pSMGFP4

(Davis and Vierstra 1998) flanked by the 5' untranslated sequence of the bacteriophage T7 gene10 and the 3' untranslated sequence of the plastid *rpl32* gene (PCR-amplified from tobacco DNA) was excised from plasmid pICF9141 with BamHI and SacII. Both fragments were ligated together into plasmid pICF9851 restricted with XhoI and SacII so that two divergent transcription units (GFP controlled by the lac-modified *rrn16* promoter and *lacI* controlled by unmodified *rrn16* promoter) were obtained. A fragment containing these transcription units was excised with SphI and XhoI and inserted after blunting of the overhanging ends with T4 DNA polymerase into the blunted SdaI restriction site of plasmid pICF9561, which contains the *aphA-6* selection marker (Huang *et al.*, 2002) provided with plastid expression signals (5'-UTR of tobacco plastid *rpl22*, 3'-UTR of *Chlamydomonas reinhardtii rbcL*), and homologous flanks for recombination with the plastome (PCR-amplified from tobacco DNA). The plastome insertion site targeted with this vector (pICF10501) is between the *trnV*(GAC) and 3'*rps12* genes of the tobacco plastome. A schematic depiction of the gene arrangement in pICF10501 is given in figure 1.

Please replace the last paragraph on page 39 with the following paragraph:

Transplastomic tobacco plants containing a recombinant GFP gene expression of which can be induced with tetracycline or anhydrotetracycline are generated by transformation with vector pICF10461. The general composition of plastid transformation vector pICF10461 corresponds to vector pICF10501 (described in example 1 and shown in figure 1), but instead of the *lacI* coding sequence the *tetR* coding sequence from transposon tn10 is inserted, and the modified *rrn16* promoter for the GFP gene contains a tet operator sequence instead of a lac operator. PCR-amplification of the tetR sequence from E. coli XL1-Blue is made with primers otet1 (SEQ ID NO:5) (5'-gaccatggctagattagataaaagtaaag-3') and otet2 (SEQ ID NO:6) (5'-cactgc agttaagaccacttcacatttaag-3'), and modification of the tobacco *rrn16* promoter by inverse PCR with primers otet3 (SEQ ID NO:7) (5'-acgtccctatcagtgatagagtattttctgggagcgaac-3') and otet4 (SEQ ID NO:8) (5'-caatcccacgagcctcttattc-3'). The cloning procedure is made analogously to vector pICF10501, so that all other regulatory elements, selection marker, and plastome insertion site are identical.

Please replace the second paragraph under Example 6 on page 42 with the following paragraph:

The riboswitch described in Suess et al. (2004) (SEQ ID NO:9) (agatgataccagccgaaaggcc cttggcagctctcg) is introduced into the 5' untranslated sequence of the bacteriophage T7 gene10 immediately upstream of the Shine-Dalgarno-sequence (AGGAG) via PCR: the sequence is included in a primer for amplification of a fragment containing of the smGFP coding sequence preceded by the Shine-Dalgarno-sequence (see example 1). A second PCR fragment consisting of the tobacco *rrn16* promoter and the rest of the T7 gene10 5'-untranslated region is amplified from plasmid pICF7341 (see example 1). Both fragments are joined via a BspMI restriction site and inserted into SdaI / AscI restricted plasmid pICF9561 (see example 1), which contains the *aphA-6* selection marker and homologous flanks for recombination with the plastome. Generation of tobacco plastid transformants is made as described in example 1. After integration into the plastome, an mRNA coding for smGFP and *aphA-6* is constitutively transcribed from the recombinant *rrn16* promoter, but translation of the GFP coding sequence is inhibited by the secondary structure in the 5'-UTR. Induction of GFP expression is made by treatment of transplastomic plants with theophylline. Immunological determination of the GFP content is made as described in example 1.

Please replace the first paragraph that begins on page 43 with the following paragraph:

A plastid transformation vector for introducing a GFP gene regulatable by this mechanism is constructed based on plastid transformation vector pICF10501 described in example 1. For replacing the T7 gene 10 5'-UTR upstream of the GFP coding sequence by the stem-loop forming 5'-UTR crR12 described in Isaacs *et al.*, 2004, the GFP coding sequence is PCR-amplified with this sequence added to the upstream primer oin1 (SEQ ID NO:10) (5'- ttggatccgaattctaccattcacctcttggatttgggtattaaaggaggagaaggtatatgagtaaaggagaagaac-3') (downstream primer oin2 (SEQ ID NO:11): 5'- tatgagctcttattgtatagttcatcatgcc -3') and inserted

into pICF10501, partially restricted with BamHI and SacI. The additional transcription unit consisting of 16Slac-promoter and the small regulatory RNA (taR12 described in Isaacs *et al.*, 2004) is added into the plasmid by insertion of a fragment made from synthetic oligonucleotides orn1 (SEQ ID NO:12) (5'- ttctggccgccgtcgtcaatgagaatg gataagaggctcgtgggattgacgattgtgagcggataacaatatattctgggagcgaac -3') and orn2 (SEQ ID NO:13) (5'- ttctggccgtctagagatatatgtagtagtaag ttaatttcattaaccaccactaccaatcacctcctggatttgggtgcgccggagttcgtcccagaaatatattg-3') into the XmaIII restriction site downstream of the operon containing lac repressor and selection marker. Generation of tobacco plastid transformants, induction with IPTG, and immunological determination of the GFP content is made as described in example 1.

Please replace the first paragraph that begins on page 44 with the following paragraph:

A plastid transformation vector for introducing a GFP gene regulatable by this mechanism is constructed based on plastid transformation vector pICF10501 described in example 1. A DNA fragment consisting of the tobacco chloroplast *rrn16* promoter, the DNA sequence for the inhibitory RNA, and the reverse 16Slac promoter, is produced by hybridizing and filling in oligonucleotides oan1 (SEQ ID NO:14) (5'- ttctggccgtcgttcaatgagaatggataagaggctcgtgggattgacgtgagggggcagggatggctatatattctgggagcgaacggaaatgctagccatatgtatatctcc -3') and oan2 (SEQ ID NO:15) (5'- ttctggccgccgtcgttcaatgagaatggataagaggctcgtgggattgacgattgtgagcggataacaatatattctgggagcgaacggagatatcatatggctagcatttcc -3') and inserted into the XmaIII restriction site of pICF10501 downstream of the operon containing lac repressor and selection marker. Generation of tobacco plastid transformants, induction with IPTG, and immunological determination of the GFP content is made as described in example 1.